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# Cytokines: IL-20 – a new effector in skin inflammation

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The newly discovered cytokine interleukin-20 (IL-20) is structurally related to IL-10, yet it appears to be an autocrine factor for keratinocytes that regulates their participation in inflammation.

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As the DNA sequences of mammalian chromosomes and vast cDNA libraries become available, researchers are vigorously scouring them for their favorite genes. The search for new cytokines has been among the most competitive and indeed one of the latest finds, interleukin-20 (IL-20), was discovered *in silico*. Using a computational method to identify sequences encoding amphipathic α helices as well as a signal peptide, Blumberg *et al.* [1] focused on a high-scoring cDNA from a keratinocyte library. This cDNA was found to encode a relative of the cytokine IL-10 and was named IL-20. Biological studies of IL-20 revealed that it has an important role in promoting hyperproliferation of keratinocytes and thereby modulating inflammation in the skin.

## A new member of the IL-10 family

The IL-20 mRNA contains motifs in its 3' untranslated region that are associated with instability. In agreement with this, the IL-20 mRNA appears to be rare and short-lived. The polypeptide sequence of IL-20 is similar (20–40% identical) to that of IL-10 and four other mammalian cytokines, as well as several viral products that constitute the IL-10 family (Table 1). The presence of IL-10-related genes in various viral genomes may reflect the ability of these factors to act as autocrine growth factors for infected cells as well as regulatory signals to parry host immune responses. Further application of sensitive algorithms to the complete sequences of the human and other mammalian genomes is likely to identify any remaining members of this family (as well as other cytokine families) in the near future.

The genes encoding the known human IL-10-related cytokines are clustered in two loci. The genes for IL-10, IL-19, IL-20 and the melanocyte differentiation-associated factor MDA-7 are found within a 200 kb region of chromosome 1, whereas genes encoding the two other family members — IL-22 and AK155 (a factor induced upon transformation by Herpesvirus) — are found within

30 kb of each other and less than 100 kb from the interferon-γ (IFN-γ) gene on chromosome 12. Certain alleles of this region of chromosome 12 have been found to be associated with asthma and inflammatory bowel disease [2]. While initial attention was drawn to the IFN-γ gene, allelic variants have not been found. Therefore it remains possible that variations in either the IL-22 or AK155 genes contribute to these diseases.

## Transgenic mice reveal skin as a major target of IL-20

To investigate the biology of IL-20 *in vivo*, several transgenic mice were generated that expressed IL-20 under the control of various tissue-specific promoters. All of these mice were runted at birth and died within days. Histological analysis revealed a profoundly thickened epidermis characterized by increased numbers of keratinocytes and expression in the suprabasal layers of differentiation and proliferation markers that are normally confined to the basal layer. These changes appeared to be caused by circulating IL-20, because even mice expressing the transgene in tissues away from the skin, such as in the liver (driven by the albumin promoter), were similarly affected.

In light of the effects of IL-20 on the epidermis of the transgenic mice, Blumberg *et al.* [1] focused their attention on keratinocytes. IL-20 was found to activate signal transducer and activator of transcription 3 (Stat3), one of the transcriptional activators involved in IL-10 signaling, in an immortalized keratinocyte cell line (HaCaT). Exposure of HaCaT cells to IL-20 induces translocation of Stat3 to the nucleus and stimulation of transcription from a Stat3-responsive reporter gene. Stat3 can be activated by more than a dozen different factors in different cell types. Its central role in development is demonstrated by the observation that Stat3-deficient mice die as early embryos. Specific deletion of the Stat3 gene in keratinocytes blocks their responses to epidermal growth factor (EGF), hepatocyte growth factor (HGF) and IL-6 [3]. Although Stat3-deficient keratinocytes can form approximately normal skin, they are defective in wound healing and have altered secondary hair cycles. While EGF is an important cytokine for keratinocytes, some of the deficits in Stat3-deficient keratinocytes may also be due to their inability to respond to IL-20, given the recent study by Blumberg *et al.* [1]. In addition, as IL-10 signal transduction includes both Stat3-dependent and -independent pathways, it is possible, by analogy, that IL-20 signal transduction is similarly complex [4].

When HaCaT cells were incubated with IL-20 in the presence of submaximal concentrations of IL-1β, tumor necrosis factor α (TNF-α) or EGF, strong cooperativity was

**Table 1**

**The IL-10 family of cytokines.**

| Cytokine | Gene location | Cellular source | Cognate receptor | Biological activities | References |
|---|---|---|---|---|---|
| IL-10 | Human 1q32 | Activated T cells, B cells, monocytes, keratinocytes | IL-10Rα, IL-10Rβ | Enhances Th2 differentiation; suppresses Th1 differentiation | [9] |
| IL-19 | Human 1q32 | Stimulated monocytes | Unknown: not IL-10Rα, IL-10Rβ | Unknown | [10] |
| IL-20 | Human 1q32 | cDNA found in a keratinocyte library | IL-20Rα, IL-20Rβ: both upregulated in psoriasis | Stimulates keratinocyte proliferation and differentiation; activates Stat3; receptor also on certain endothelial and mononuclear cells | [1] |
| MDA-7 (mob-5, C49a) | Human 1q32 | Melanocytes, stimulated cells, melanoma cells, fibroblasts in wound repair, ras-transformed cells | Binding activity detected on ras-transformed cells | Expression associated with differentiation of melanocytes; reverts malignant phenotype of melanoma; upregulated in wound healing; autocrine factor of ras-transformed cells | [11–13] |
| IL-22 (IL-TIF) | Human 12q15 | IL-9- or lectin-stimulated T cells, mast cells | IL-22Rα, IL-10Rβ | Activates Stat1, 3, 5; stimulates monocytes to make TNF | [14–16] |
| AK155 | Human 12q15 | Herpesvirus saimiri transformed T cells | Unknown | Transformation of T cells? | [17] |
| ebvIL-10 (BCRF1) | Epstein–Barr virus (EBV) genome | EBV-infected cells | IL-10Rα, IL-10Rβ | Similar to IL-10 but lower affinity; may block immune response to viral infected cells; autocrine growth factor for infected B cells. | [18,19] |
| cmvIL-10 | Cytomegalovirus (CMV) genome | CMV-infected cells | IL-10Rα, IL-10Rβ | Similar to IL-10, may block immune response to virus-infected cells | [20] |
| hpvIL-10 | Herpesvirus papio genome | Unknown | Unknown | Unknown | Genbank accession AAF23949 |
| yldvIL-10 | Yaba-like disease virus genome | Unknown | Unknown | Unknown | Genbank accession NP_073519 |

observed, increasing their sensitivity to IL-20 by as much as 10-fold and quadrupling the magnitude of their response. Three genes found to be upregulated by IL-20 in HaCaT cells encode proteins involved in inflammation: TNF-α, which is likely to further enhance the response of keratinocytes to IL-20 and also stimulates chemotaxis and antimicrobial activity of myeloid cells; the chemokine MCP-1, involved in leukocyte chemotaxis; and MRP-14, one of the S100 calcium-binding proteins implicated in neutrophil integrin activation and associated with epidermal inflammation [5].

### The IL-20 receptor is upregulated in psoriasis

Receptor chains expressed in HaCaT cells were examined and two previously known but orphan class II cytokine receptor chains (now named IL-20Rα and IL-20Rβ) were found to form a functional cognate receptor for IL-20

when co-transfected into a different cell line. Because suprabasal expression of basal keratins and proliferation markers, as well as certain structural alterations in the epidermis of the IL-20 transgenic mice are similar to features of psoriasis, expression of the two receptor chains was measured in normal and psoriatic human skin. While very low levels were detected in normal skin, dramatic induction of both chains was found in keratinocytes, endothelial cells and certain mononuclear cells in psoriatic lesions. The hyperproliferation of keratinocytes associated with psoriasis is dependent upon the participation of activated T cells [6]; IFN-γ, normally a product of activated T cells, provokes similar hyperproliferation of keratinocytes [7]. Therefore it seems likely that expression of the IL-20 receptor, and perhaps IL-20, by keratinocytes is stimulated by IFN-γ or other products of activated T cells. Whether in concert with other factors or expressed by

**Figure 1**
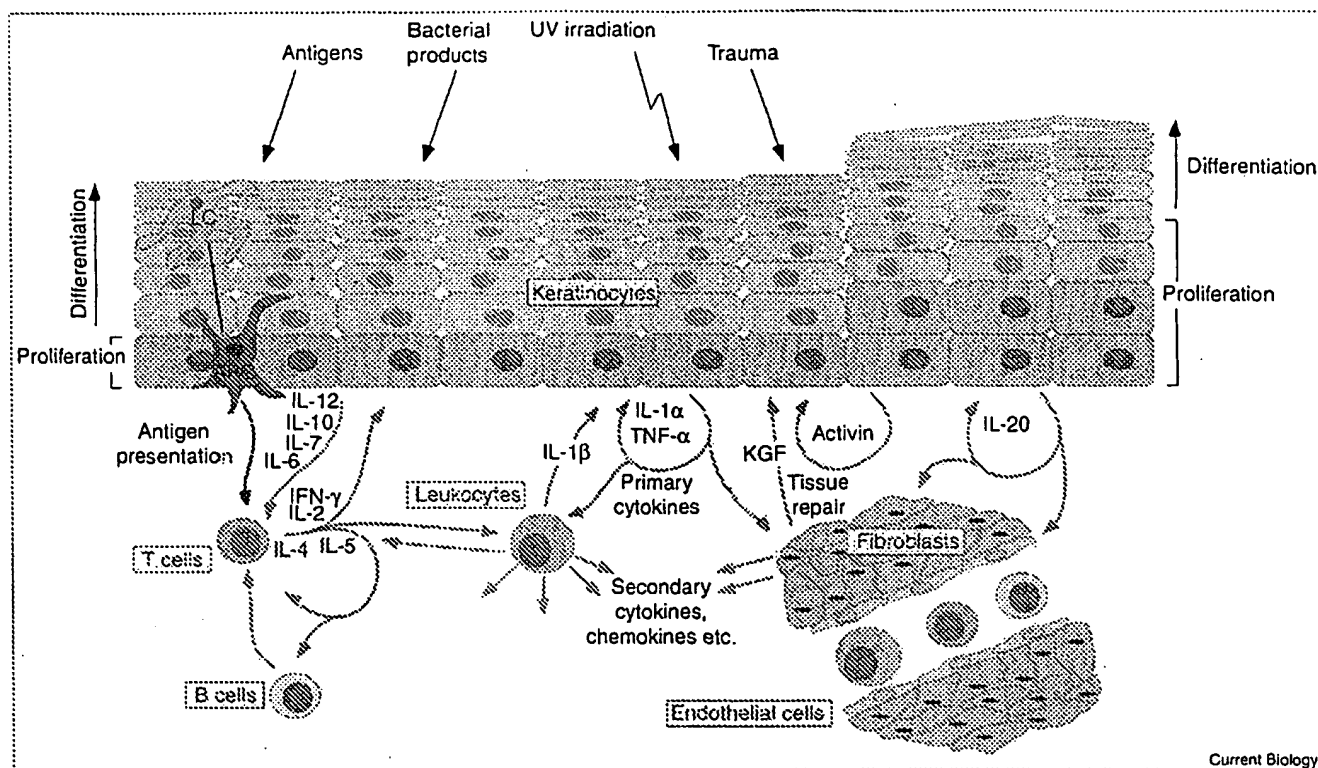


Signaling pathways in inflammation of the skin. Basal layers of keratinocytes proliferate while upper levels differentiate to form the barrier. Changes in proliferation and differentiation alter the structure of the skin. External stimuli such as trauma or irradiation cause keratinocytes to release primary cytokines, IL-1α and TNF-α. These factors provoke fibroblasts, leukocytes and keratinocytes to express more primary cytokines (IL-1) as well as proinflammatory secondary cytokines and chemokines. Trauma also elicits KGF and activin expression which promote repair. Foreign antigens are captured by Langerhan's cells (LC) and other antigen-presenting cells (APC) which migrate and interact with T cells. Activated T cells, supported by secondary cytokines from the epidermis, dermis and leukocytes, proliferate and release cytokines that promote cellular (Th1, involving IFN-γ) or humoral (Th2, involving IL-4) immune responses. Signals that control IL-20 expression are unknown, however IL-20 receptor is upregulated in psoriatic epidermis, as well as some leukocytes and endothelial cells.

itself, IL-20 exerts distinctly proinflammatory effects on keratinocytes and may play a central role in the epidermal response to inflammation.

**Two functions of the epidermis**

When investigating the role of cytokines in the epidermis, we must consider that skin has two fundamental functions: first, it provides a self-repairing physical barrier to keep the rest of the organism in and the outside world out, and second, it acts as a dynamic environment for the front line of the immune system to encounter and respond to pathogens. These two functions act in concert to protect the organism from the environment. Figure 1 illustrates some of the signaling pathways involving the epidermis. Several different circumstances can lead to changes in the homeostasis of the epidermis and structural perturbations. Some phenomena such as physical trauma, ultraviolet radiation or bacterial lipopolysaccharide have direct effects on keratinocytes and underlying fibroblasts, triggering the innate immune

system, whereas others such as antigenic challenges are mediated by cells of the acquired immune system.

**Differentiation vs proliferation in the epidermis**

The outermost layer of skin, the epidermis, consists of layers of keratinocytes stratified in a gradient of differentiation (Figure 1). Keratinocytes progress through a program of differentiation as they move up through the skin to become corneocytes, dead bundles of precipitated keratin proteins wrapped in remnants of plasma membrane. These protein 'bricks' and lipid 'mortar' form the barrier of the skin called the stratum corneum. While cornified cells are continually sloughed off, the less-differentiated keratinocytes of the innermost basal layer of the epidermis proliferate and differentiate as they are forced upwards, continually reforming the barrier. Consequently, the structure of the epidermis is exquisitely dependent upon the homeostatic balance between proliferation and differentiation by the viable keratinocytes in the lower layers of the

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epidermis. One of the keys to understanding the biology of the skin is deciphering the molecular events that influence the keratinocytes' decision between proliferation and differentiation. Signals originating in the dermis, including keratinocyte growth factor, are amplified by induced expression of activin, and alter this balance during wound repair and development [8]. IL-20 appears to be a signal originating in the epidermis that directly impacts this decision in the context of inflammation.

## Activation of keratinocytes

In addition to altering the balance between proliferation and differentiation, keratinocytes participate in immune responses by releasing signaling molecules. The ability of keratinocytes to release complex arrays of proinflammatory factors when provoked by stimuli such as physical trauma, ultraviolet irradiation, bacterial products or cytokines allows them to recruit inflammatory cells and regulate their behavior. Factors released by keratinocytes in response to various stimuli include TNF-$\alpha$, IL-1$\alpha$, IL-3, IL-6, IL-7, IL-8, IL-10, TGF-$\alpha$, TGF-$\beta$, IFN-$\gamma$ and MCP-1 among others. It is not clear whether these are products of a single type of keratinocyte activation or derive from several qualitatively different responses. In either case, these factors convey signals in a paracrine fashion to other cells including leukocytes, endothelial cells and fibroblasts as well as in an autocrine fashion to the keratinocytes themselves. Two prominent autocrine factors for keratinocytes, TNF-$\alpha$ and IL-1$\alpha$, are termed primary cytokines because they activate the NF$\kappa$B pathway, stimulating transcription of a number of proinflammatory cytokines, chemokines, adhesion molecules and other effectors in many cell types.

The biology of IL-20 must be viewed within the context of this already crowded milieu of intercellular signals involved with cutaneous inflammation and homeostasis. Among these signals, however, there is no clear pathway by which activated T cells can trigger the release of primary cytokines by keratinocytes and the ensuing cascade of proinflammatory events in the skin. The prominent expression of the IL-20 receptor in psoriatic skin hints that IL-20 expression may be an important step in this process.

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